RESPONSE TO LIGHT IN PERANEMA TRICHOPHORUM WITH SPECIAL REFERENCE TO DARK-ADAPTATION AND LIGHT-ADAPTATION

By

LANDRUM B. SHETTLES

A DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Baltimore, Md.
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RESPONSE TO LIGHT IN PERANEMA TRICHOPHORUM WITH SPECIAL REFERENCE TO DARK-ADAPTATION AND LIGHT-ADAPTATION

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TWELVE FIGURES

INTRODUCTION

Mast ('12) and Mast and Hawk ('36) observed in Peranema trichophorum that if the luminous intensity is rapidly increased, there is an increase in the rate and the extent of vibration of the flagellum as well as in the portion which vibrates, that this is followed by sharp deflection of the anterior end of the body through an angle of nearly 180°, after which the body and the flagellum straighten somewhat, the tip becomes active, and the creature proceeds on a new course at an angle of about 90° with the old. They found that if the luminous intensity is slowly increased, no response occurs, i.e., that the response depends upon the rate of change in luminous intensity and is therefore a typical shock reaction. They maintain that "if light-adapted specimens of Peranema trichophorum are subjected to darkness, the sensitivity to light increases rapidly to maximum (as the time in darkness increases), then decreases slowly to minimum after which it remains nearly constant," and that "the sensitivity reaches maximum after about 1 hour in darkness and minimum after about 4 hours."

[1] I am very much indebted to Prof. S. O. Mast who suggested the problem and made valuable suggestions and criticisms during the course of the experimental observations and the preparation of the manuscript, and to Prof. A. H. Pfund of the department of physics of the Johns Hopkins University for invaluable assistance in the work on wave lengths and the loan of apparatus and laboratory facilities used in this work.

The present paper constitutes an extension of the work of Mast and Hawk.

MATERIAL

Peranema trichophorum is a colorless flagellate without an eye spot. It is pointed at the anterior end and truncate at the posterior and varies in length from about 25 to 70 μ. It is highly plastic, especially when it changes direction of locomotion. The flagellum is a thick structure, ranging from about one-fourth to almost the length of the body.

Mast ('12) observed two methods of locomotion in Peranema, a gliding method and a free swimming one. He says that with the latter it swims through the water very much like Euglena; with the former it "proceeds with a given surface continuously in contact with the substratum to which it adheres with considerable tenacity, probably by means of secreted mucus," and that the flagellum extends straight forward, "rigid and motionless except for about one-sixth of the whole at the tip, which is bent at right angle so as to form a sort of hook" and vibrates in such a way that the free end describes an ellipse. All the observations considered in the following paper were made on peranemas moving by the gliding method.

The fact that the rate of locomotion is comparatively slow makes it easy to follow the activities of the organism even under high magnification. Moreover, its response to stimulation is marked and precise. It is therefore very favorable for quantitative studies concerning responses.

METHOD OF CULTURE

Peranema is frequently found in laboratory cultures, especially those which are rich in decaying organic matter. The peranemas used in the present investigation were obtained from cultures which were essentially the same as those used for Amoeba proteus by Hahnert ('32). They consisted of salts of the highest purity, water redistilled in Pyrex glass, polished rice, and chilomonads in the following proportions:

NaCl, 0.08 gm.; $NaHCO_3$, 0.004 gm.; KCl, 0.004 gm.; $CaCl_2$, 0.004 gm.; $CaH_4(PO_4)_2$, 0.002 gm.; Na_2HPO_4, M/15, 2 cc.; KH_2PO_4, M/15, 3 cc.; redistilled water, 1000 cc.; rice, 4 grains, and old culture fluid containing chilomonads, 1 cc. per 150 cc.

Finger bowls, each containing about 150 cc. of this solution, were covered, put into a light-tight chamber, and left about a week; then peranemas were added. The rice soon became covered with mold, on and among the strands of which numerous peranemas and chilomonads aggregated. These cultures remained in a flourishing condition for a month or more. Throughout the entire work the organisms used were cultured in total darkness, and they were therefore fully dark-adapted.

DISTRIBUTION OF PHOTOSENSITIVE SUBSTANCE IN PERANEMA

The apparatus and the methods used in making observations on the distribution of photosensitive substance in Peranema were essentially the same as those used by Mast ('32 a) in making observations on localized stimulation, transmission of impulses, and the nature of response in Amoeba. A monoplane-filament stereopticon lamp was mounted in a light-tight black box with an opening 2 cm. square. A heat filter, 4 cm. thick, filled with distilled water, was mounted in front of the opening in the box. A piece of black tin containing a small slit was placed over the opening in the box and adjusted so that only a small portion of one section of the filament in the lamp was exposed. This produced a sharp, clear-cut area of light 0.015 mm. wide and 0.1 mm. long. The light from this area was reflected by means of the plane substage mirror up through a 4 mm. objective used as a condenser in the substage of the microscope. This produced an image with sharp clear-cut edges, which could be moved in any direction in the plane of the stage of the microscope by rotating the mirror, and it could be focused by raising or lowering the substage condenser. The lamp was 30.5 cm. from the center of the stage on the microscope.

The observations were made as follows: A dark-adapted specimen was isolated in a drop of filtered culture fluid on a slide under a cover glass supported with a vaseline ring. Several slides were prepared in this manner. They were then exposed in diffuse daylight and left 2 hours, after which one was placed on the stage of the microscope in diffuse daylight in front of a north window. The lamp was then turned on and so adjusted that the image of the filament was in the center of the stage. The image was then brought to sharp focus. After the specimen was located, the slide and the image were moved about by means of a mechanical stage and the substage mirror so as to bring the tip of the flagellum into the field of light. The time required for a response (reaction time) was then measured with a stop watch, after which the lamp was turned off; then the specimen was left in diffuse daylight 3 minutes, after which the lamp was again turned on; then the slide and the image were moved so as to bring the anterior end of the body (but not the flagellum) into the field of light, and the reaction time measured. This was then repeated for the posterior end. The specimen was then discarded, after which the whole process was repeated with four other specimens, except that the order of observation on the three regions was changed.

The response was precisely the same in character no matter which of the three parts was illuminated. There was no indication whatever of a differential response to localized stimulation.

In peranema A when the tip of the flagellum was illuminated first, the anterior end of the body second, and the posterior end third, the reaction time was 27.2, 31.0 and 33.5 seconds, respectively, and similar results were obtained for peranema D. In peranema B in which the tip of the flagellum was illuminated second, the anterior end third, and the posterior end first, the reaction time was 24.3, 30.1 and 34.2 seconds, respectively. Similar results were obtained for peranema E, and in peranema C in which the tip of the flagellum was illuminated third, the anterior end second, and the posterior

end first, the reaction time was 20.0, 28.4 and 31.7 seconds, respectively.

The results therefore show that no matter in which order the three regions are illuminated, the reaction time is shortest for the tip of the flagellum and longest for the posterior end of the body.

From these results it is concluded that the entire animal is sensitive to light, and that the flagellum is most sensitive, the anterior end less, and the posterior end least.

If the size of the flagellum is taken into consideration in comparison to that of either the anterior or the posterior end of the body, it becomes evident that the flagellum is per unit volume very much more sensitive than the other regions.

RESPONSE TO LIGHT OF THE DETACHED FLAGELLUM OF PERANEMA

The observations on the response to light of the detached flagellum of Peranema were made as follows: A Peranema culture was taken from total darkness, placed in diffuse daylight near a north window, and left there 2 hours. Then a specimen was taken from the culture and mounted on a slide on the stage of the microscope in diffuse daylight in a drop of filtered culture fluid surrounded by a vaseline ring. Then under a 10X dry objective and a 10X compensating ocular the flagellum was detached by means of a fine glass rod, after which it was left some time, then suddenly exposed to strong light from a 165-watt lamp, and observed under a 44X dry objective and the 10X compensating ocular. In this way thirteen flagella were studied. The results obtained were essentially the same for all. Those obtained for four are recorded in figure 1.

After flagellum A was cut from the body of the peranema, it was left in weak light 300 seconds. During this time there was no change in shape (fig. 1 A, 1–5). It was then exposed to strong light 15 seconds. At the close of this time there was a marked bend at both ends (A, 6). It was then subjected to weak light 240 seconds. During this time there was no change in shape (A, 7–8).

Flagellum B was left in weak light 240 seconds. During this time there was no change in shape (fig. 1 B, 1–4). It was then exposed to strong light 15 seconds. At the end of this time there was a definite change in shape throughout (B, 5). It was then subjected to weak light 30 seconds and then exposed to strong light 15 seconds. At the close of this time there was a further change in shape throughout, but not so great as the former (B, 6).

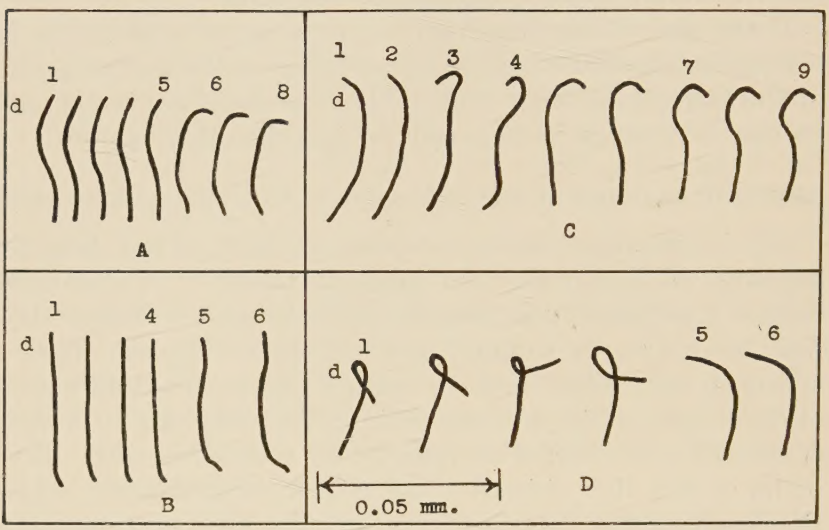

Fig. 1 Camera lucida drawings illustrating response to light of the detached flagellum of Peranema trichophorum. A, B, C, D, four flagella; d, distal end.

Flagellum C was left in weak light 300 seconds. During this time there was no change in shape (fig. 1 C, 1–2). It was then exposed to strong light 15 seconds. At the end of this period there was a marked bend at the distal end (C, 3). It was then subjected to weak light 30 seconds and to strong light 15 seconds. At the end of this exposure there was a change in shape throughout (C, 4). This was repeated again, and it straightened considerably except for a bend in the distal region (C, 5). This was repeated a third and fourth time, and similar results were obtained (C, 6–7). It was then subjected to weak light 240 seconds. During this time there was no further change (C, 8–9).

Flagellum D was exposed to strong light 15 seconds and then to weak light 30 seconds in successive tests. At the end of the respective exposures there were definite changes in the distal part (fig. 1 D, 1–5), especially marked in the last test (D, 5). It was then subjected to weak light 300 seconds. During this time there was no further change in shape (D, 6).

The results therefore show that the detached flagellum does not change in shape in weak light no matter how long it is exposed, that it responds by change in shape if the intensity of the light is rapidly increased, but that after it responds to light it does not recover either in strong or in weak light.

From these results it is concluded that the detached flagellum is sensitive to increase in illumination, and that it possesses the ability to respond but not the ability to recover.

DISTRIBUTION OF STIMULATING EFFICIENCY IN THE VISIBLE SPECTRUM

The literature on the distribution of stimulating efficiency in the spectrum was, in reference to lower organisms, thoroughly reviewed by Mast ('17) and Bertholf ('32). These reviews show that unequal distribution of energy in the spectrum and unequal energy of colors produced by filters was adequately considered in only a few of the investigations.

Gross ('13) measured both the wave length of the light and the energy, and he compared the stimulating efficiency of four spectral zones of equal energy in adults of Drosophila ampelophila, Feltia subgothica, Calliphora erythrocephala, and Periplaneta americana, and in larvae of Zuerzera pyrina and Calliphora erythrocephala. He concludes that for each of these the most efficient wave lengths are between 430 and 490 mμ.

Mast ('17) used a Hilger spectrometer and a Lummer-Brodhun rotating sector, made corrections for unequal distribution of energy, and ascertained with great accuracy the relative stimulating efficiency at intervals of 10 mμ throughout the visible spectrum, and he found from the study of fifteen species, including unicellular and colonial forms, worms, and fly larvae, that the maximum stimulating efficiency is at wave

length 483 mμ for Euglena viridis, Euglena gracilis, Euglena tripteris, Euglena granulata, Euglena minima, Trachelomonas euchlora, Phacus triqueter, Gonium pectorale, Arenicola cristata, and Lumbricus terrestris; wave length 524 mμ for Pandorina morum, Eudorina elegans, and Spondylomorum quaternarium; and wave length 503 mμ for Chlamydomonas (fluvialis and globulosa?) and blowfly larvae, and that from these regions it decreases very rapidly toward either end of the spectrum, but that there is no evidence indicating that stimulation is independent of luminous intensity.

Laurens and Hooker ('20) studied the stimulating efficiency of light at intervals of 20 mμ from wave length 420 to 660 mμ but of equal energy in Volvox globator and concluded that wave length 494 mμ has the highest stimulating value.

Visscher and Luce ('28) tested the effect of thirteen spectral zones of equal energy on the cyprid larvae of Balanus improvisus and Balanus amphitrite. They maintain that the region around 540 mμ is most efficient.

Bertholf ('31, '32) made observations on Apis and Drosophila melanogaster using essentially the same methods as Mast did. He found that, as the wave length decreases, the stimulating efficiency for Apis increases from 0 at about wave length 645 mμ to a maximum of 100 arbitrary units at 555 mμ, then decreases to 10 at 534 mμ, after which it rapidly increases to a second maximum of 450 at 365 mμ, and then decreases to 0 at about 280 mμ, and that the distribution of stimulating efficiency for Drosophila is essentially the same.

The experiments on the distribution of stimulating efficiency in the visible spectrum for Peranema were made in a dark room in which the temperature was continuously $23 \pm 0.5°$. The apparatus used is represented in figure 2. Monochromatic light was obtained by use of a monochrometer which consisted essentially of a brass jacket (W_1) with a slit (S_2) at one end through which the light entered and the width of which was adjusted by a screw (S_1) in regulating the intensity, a slit (S_3) fixed in width at the other end through which the light emerged, two lenses (L_2, L_3), and a micrometer screw (S) by means of

which two prisms (P_1, P_2) were adjusted so as to obtain a desired wave length. A 15-watt, 8-volt lamp (L) in a circuit maintained at 8 volts by means of variable resistance was used as a source of light. The lamp was mounted in a black box (D) which was ventilated. A beam of light from an aperature (A) 2 cm. square, which passed through lens (L_1), a

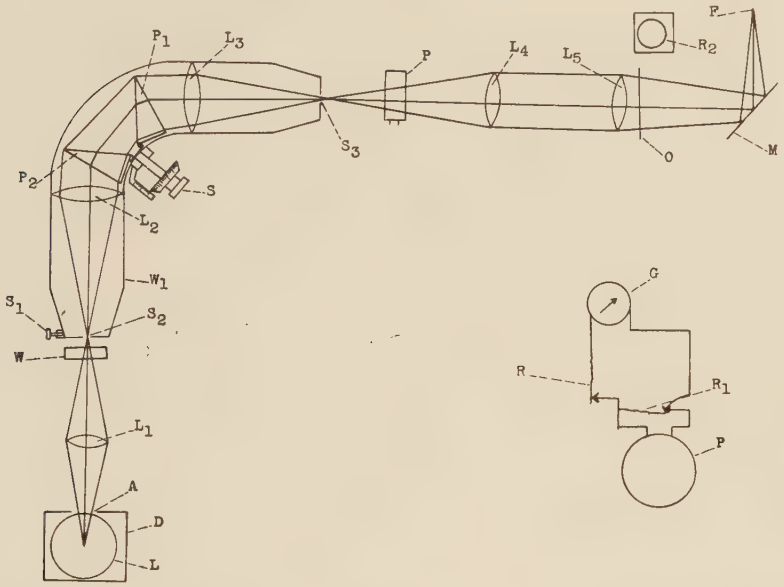

Fig. 2 Diagram of apparatus used in experiments on distribution of stimulating efficiency in the visible spectrum. L, 15-watt, 8-volt lamp; L_1, L_2, L_3, L_4, L_5, lenses; W, heat filter; S, micrometer screw; S_1, screw for adjusting intensity; S_2, S_3, slits in monochrometer; P, photronic cells; P_1, P_2, prisms; M, substage mirror; F, point of focus of light on slide; O, shutter; G, galvanometer; R, series resistance-5000 ohms; R_1, shunt resistance-900 ohms; W_1, wall of monochrometer; R_2, ruby lamp; D, black box; A, aperture in black box.

heat filter (W), the monochrometer, and lenses (L_4, L_5), was reflected by the plane substage mirror (M) to the stage of a compound microscope, which had no substage condenser. The light from a movable ruby lamp (R_2), placed in front of the microscope, was also reflected by the substage mirror. A shutter (O) was placed in front of the substage mirror.

A movable photronic cell (P), connected to a galvanometer (G) in a series resistance of 5000 ohms (R) and in a shunt resistance of 900 ohms (R_1), was placed in the beam of light between slit (S_3) and lens (L_4). The photronic cell was calibrated against a thermocouple so as to ascertain its sensitivity for light of different wave lengths but of equal intensity, as follows: With the 15-watt lamp on, slit (S_2) open, screw (S) set for 400 mμ, and a thermocouple connected to a galvanometer in the position of the photronic cell, the deflection of the galvanometer was 116 mm. (designated B). Then the thermocouple was replaced by the photronic cell. The deflection of the galvanometer was now 126 mm. (designated A). This was then repeated with the screw (S) set for 428, 458, 477, 500, 516, 538, 560, 582, 612, 648, and 687 mμ, respectively, and the slit (S_2) adjusted so that the deflection of the galvanometer with the thermocouple in the beam of light was 116 mm. for each wave length tested. Then for each wave length the quotient of A/B was calculated, e.g., for wave length 400 mμ A = 126, B = 116 and A/B = 1.09. Then each quotient was divided by the largest quotient (2.78), making the largest sensitivity value of the photronic cell equal to 1. The results obtained are presented in figure 3.

The observations were made as follows: A dark-adapted specimen was mounted on each of several slides in a drop of filtered culture fluid under a cover glass supported with a vaseline ring. Then one of the slides was transferred to the stage of the microscope, with the 15-watt and the ruby lamps on, the shutter (O) closed, slit (S_2) open, and the screw (S) set for 400 mμ. Then the specimen was brought to focus in the red light, after which it was suddenly exposed to light of wave length 400 mμ, and the reaction time measured. This process was then repeated with nine other specimens. The whole process was now repeated with the intensity of the light decreased to one-half and one-fourth, respectively, by putting wire gauze across the beam of light. Then it was repeated with the wave length of the light 420, 440, 460, 480, 490, 500, 505, 510, 520, 540, 560, 570, 575, 580, 585, 600, and 605 mμ, respectively, but equal in intensity.

The intensity was made equal as follows: It will be seen from the curve in figure 3 that, e.g., at wave length 420 mμ the sensitivity of the photronic cell was 0.47, and at wave length 400 mμ it was 0.39. To make the intensity with wave length

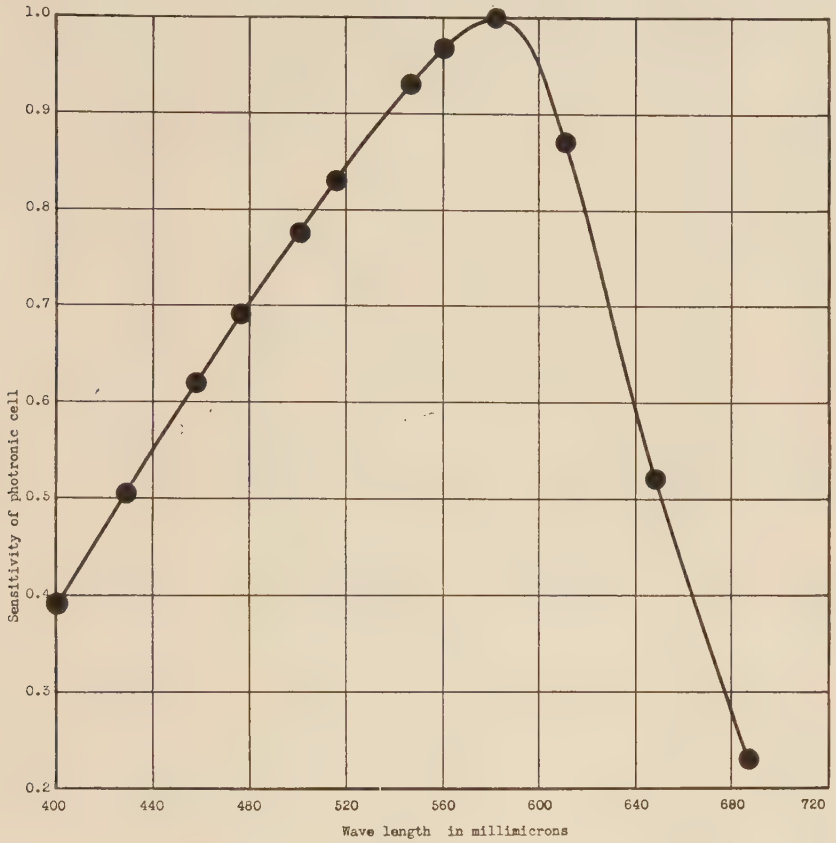

Fig. 3 Graph showing relation between sensitivity of photronic cell and wave lengths of equal intensity. The points on the curve represent the sensitivity values.

420 mμ equal to the intensity with wave length 400 mμ, it was therefore only necessary to put the photronic cell into the beam and adjust the width of slit (S_2) until the deflection of the galvanometer was 47/39 of 126 or 152 mm.

TABLE 1

Relation between wave length and reaction time. Dark-adapted peranemas were exposed to light of different wave lengths, intensity 4X m.c., and the reaction time measured. Each reaction time presented is the result of one observation on one individual, and different individuals were used in each observation

DESIGNATION OF PERANEMAS	WAVE LENGTH IN MILLIMICRONS														
	400	420	440	460	480	490	500	505	510	520	540	560	580	600	605
	REACTION TIME IN SECONDS														
1	38.3	25.3	21.0	11.3	8.3	6.3	4.9	4.6	5.1	9.5	16.7	21.4	35.3	53.1	73.1
2	42.1	30.2	17.4	15.6	10.4	7.8	5.6	3.0	4.9	10.6	12.9	25.6	48.4	55.2	68.7
3	36.7	27.8	19.6	13.9	6.7	5.6	3.4	4.1	5.0	11.2	10.2	20.7	40.1	60.0	59.4
4	48.0	29.4	26.4	9.8	9.2	6.6	4.7	2.9	3.7	6.3	13.7	19.8	39.6	62.7	67.7
5	40.2	19.5	18.2	16.4	8.9	9.7	4.0	3.6	6.2	8.7	17.4	30.6	50.2	59.6	65.2
6	29.6	22.8	15.9	12.8	7.0	4.9	5.3	3.1	5.8	8.3	15.0	28.1	46.0	65.8	76.3
7	41.4	24.0	18.7	14.0	13.5	10.3	5.0	2.9	4.6	12.4	9.8	25.0	31.9	48.7	63.7
8	50.3	35.8	20.2	12.5	6.2	8.4	3.5	2.8	7.0	7.9	18.4	22.9	37.3	70.1	75.8
9	39.9	17.2	18.5	17.6	7.3	6.7	4.2	3.0	4.1	14.1	12.3	20.7	47.8	67.5	55.0
10	44.2	28.7	23.3	10.1	9.0	5.9	6.4	3.4	4.0	7.4	14.0	17.5	43.0	56.9	63.2
Total average	41.07	26.07	19.92	12.40	8.65	7.22	4.70	3.34	5.04	9.64	14.04	23.23	41.96	59.96	66.81
Probable error	1.11	1.04	0.56	0.50	0.51	0.34	0.17	0.11	0.19	0.46	0.54	0.68	1.17	1.28	1.36

Dark-adapted peranemas exposed to light of wave lengths above 605 mμ did not respond. Light of wave lengths below 400 mμ was not tested. The results obtained are presented in table 1 and figure 4.

Table 1 shows that there was remarkably little variation in the reaction time observed in the different individuals used, the probable error being only 0.11 to 1.36. Figure 4 shows that in

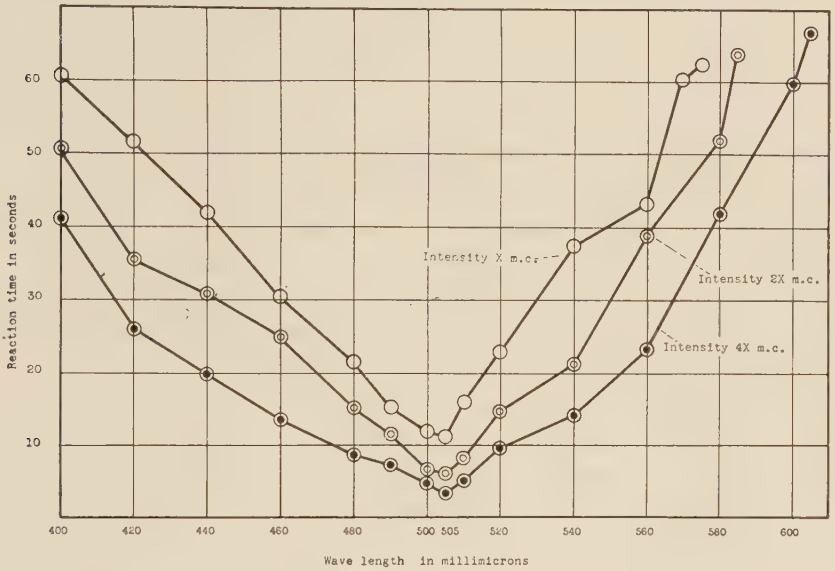

Fig. 4 Graph showing distribution of stimulating efficiency in the visible spectrum. Dark-adapted peranemas were exposed to light (intensity given in the graph) of different wave lengths, and the reaction time measured. Each point on the curves represents the average reaction time for ten tests, different individuals being used in each test.

the highest intensity used (4X m.c.), as the wave length increased, the reaction time decreased from 41.07 seconds at 400 mμ to a minimum of 3.34 seconds at 505 mμ, and then increased to 66.81 seconds at 605 mμ, that in the lower intensities, X and 2X m.c., respectively, the relation between wave length and reaction time was essentially the same as in 4X m.c., but that the reaction time was longer at all wave lengths, and that the effective wave lengths did not extend so far toward the red end of the spectrum.

The results therefore show that the maximum stimulating efficiency in the visible spectrum is approximately at wave length 505 mμ, that stimulation by light is dependent upon the wave length as well as upon the intensity, and that the limits of the spectrum are dependent upon the intensity.

RELATION BETWEEN LIGHT-ADAPTATION AND SENSITIVITY

The experiments on the relation between light-adaptation and sensitivity were made in a basement dark room in which the temperature varied from 21 to 23.5°. Peranema was adapted to light in a chamber which contained a 200-watt, 120-volt lamp on a 120-volt circuit. The lamp was attached to the center of the top of the chamber which was covered with black paper and ventilated. A heat filter, filled with distilled water to a depth of 15 cm., was mounted just below the lamp. A glass plate, 30 × 30 cm., was fastened to a ringstand so that it could be raised or lowered and the intensity of the light on it changed. The intensity of the illumination was measured by means of a Weston photometer.

The reaction time was ascertained by means of apparatus which was essentially the same as that used by Mast and Hawk ('36). This apparatus contained a 1000-watt, 120-volt projection lamp in a circuit maintained at 110 volts by means of variable resistance. The lamp was mounted in a light-tight shaft which was ventilated. A beam of light from an aperture 2 cm. square was reflected by the plane substage mirror up through the stage of a compound microscope, which had no substage condenser. The light from a movable ruby lamp, placed in front of the microscope, was also reflected up through the stage by the substage mirror. The microscope was on a guide rack on which it could be moved nearer to or farther from the source of light, thus making it possible to obtain various intensities. The intensity of the light was measured by removing the stage of the microscope and putting the target of the photometer horizontally in the position of the stage over the reflected beam of light from the plane substage mirror.

A shutter and a heat filter, 4 cm. thick, filled with distilled water, were mounted in the beam of light in front of the opening in the ventilating shaft. The light was flashed on or off by means of the shutter in which there was a sliding panel which contained two opaque plates with an opening 3 cm. wide between them. This panel was mounted in a grooved frame so that it slid up and down freely, and it had a 50-gm. weight attached to the bottom to increase the rate of fall. Two cords extended from the panel to the microscope, one of which was used to raise the panel, the other to lower it. A stop watch was connected with the latter in such a way that the panel was released and the stop watch started simultaneously.

The observations were made as follows: Some fluid was taken from a dark-adapted Peranema culture and filtered; peranemas were then taken from this culture in red light, and one mounted on each of several slides in a drop of the filtered fluid under a cover glass supported with a vaseline ring. Then the slides were put into the adapting chamber in light of 538 m.c. and left $\frac{1}{12}$ hour. Then one of the slides was put into a light-tight box and left $\frac{1}{2}$ hour, after which it was transferred to the stage of the microscope, with the 1000-watt and the ruby lamps turned on, but the panel raised so as to intercept the light from the 1000-watt lamp. Then the specimen was brought to focus in the red light, after which it was suddenly exposed to 2152 m.c. from the 1000-watt lamp by releasing the panel, and the reaction time measured. Then it was discarded. This process was repeated with the other peranemas on the slides after having been exposed to 538 m.c. $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 3 and 4 hours, respectively. This was repeated nine times with other specimens. Then the whole process was repeated with the intensity of the light in the adapting chamber 2152, 4305, 6458, 8611, and 10,764 m.c., respectively. The results obtained are presented in figure 5.

This figure shows that after dark-adapted peranemas had been adapted to light of a given intensity for different periods of time and then subjected to darkness $\frac{1}{2}$ hour, the reaction time in 2152 m.c. decreased to a minimum, as light-adaptation increased, then increased to a maximum, and then remained

nearly constant, and that about ½ hour of light-adaptation was required for the minimum reaction time no matter what the intensity in which they were adapted was, but that for each period of light-adaptation tested the reaction time varied inversely with the adapting intensity.

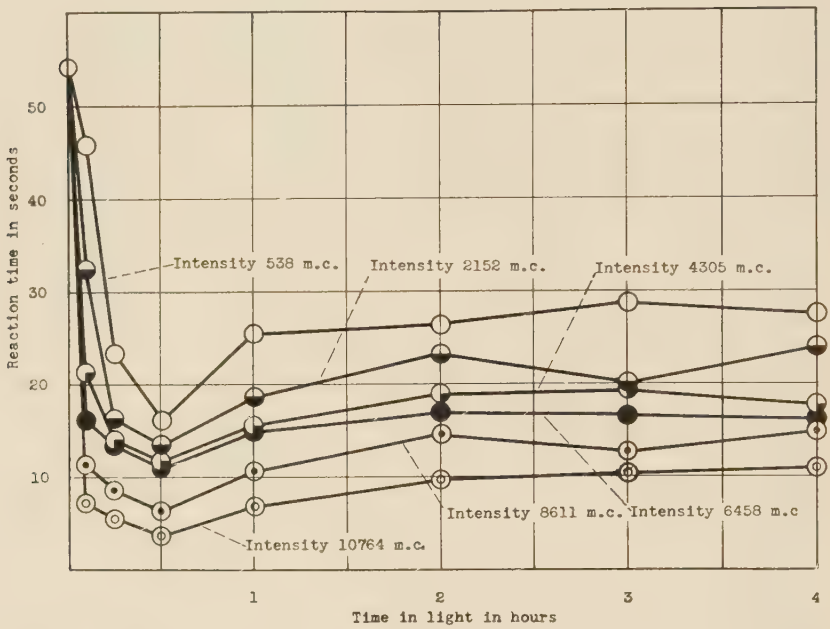

Fig. 5 Graphs showing rate of light-adaptation. Dark-adapted peranemas were exposed to light (intensity given in the graph) for the time indicated, then subjected to darkness ½ hour, then exposed to 2152 m.c., and the reaction time measured. Each point on the curves represents the average reaction time for ten tests, different individuals being used in each test.

These results indicate therefore that as dark-adapted peranemas become light-adapted their sensitivity to light increases to a maximum, then decreases considerably, and then remains constant.

RELATION BETWEEN DARK-ADAPTATION AND SENSITIVITY

Results obtained by Hecht ('18, '19, '27) for Mya arenaria, Pholas dactylus, Ciona intestinalis, and Homo sapiens; Obreshkove ('21) for Rana clamitans; Folger ('25) for Amoeba

proteus; Crozier and Wolf ('28) for Agriolimax campestris; Hartline ('30) for Limulus polyphemus, and Wolf and Wolf ('35) for Apis indicate that if photosensitive animals are subjected to darkness, the sensitivity to light increases to a maximum, as dark-adaptation increases, and then remains constant.

Results obtained by Dolley ('29) for Eristalis tenax, Mast ('32) for Volvox globator, and Mast and Hawk ('36) for Peranema trichophorum indicate that if photosensitive animals are subjected to darkness, the sensitivity to light increases to a maximum, as dark-adaptation increases, then decreases to a minimum, and then remains nearly constant.

Two methods were used to ascertain the relation between dark-adaptation and sensitivity in Peranema.

1. Dark-adapted specimens were exposed in 2152 m.c. 3 hours, then subjected to darkness ¼, ½, 1, 2, 3, 4, 5, 6, 12 and 24 hours, respectively, after which the reaction time in 538, 2152, 3229, 4305, 6458 and 10,764 m.c., respectively, was measured, different individuals being used in each observation as in the preceding experiments. The results obtained are presented in figure 6.

This figure shows that as the time in darkness increased, the reaction time, in all the intensities tested, decreased to a minimum in about 1 hour, then increased to a maximum in about 3 hours more, and then remained nearly constant, and that for all the periods of dark-adaptation tested the reaction time varied inversely with the intensity inducing the response.

These results seem to prove therefore that as adaptation to darkness progresses, the sensitivity of Peranema to light increases to maximum in about 1 hour, then decreases to minimum in about 3 more hours, and then remains constant, and that about 4 hours in darkness neutralize the effect of 3 hours in light of 2152 m.c., i.e., that it requires about 4 hours in darkness for Peranema to become fully dark-adapted after it has been in light of 2152 m.c., 3 hours.

2. Dark-adapted specimens were exposed 3 hours in light of 538, 2152, 4305, 6458 and 8611 m.c., respectively, and then subjected to darkness ¼, ½, 1, 2, 3, 4 and 5 hours, respectively,

after which the reaction time was measured in 2152 m.c. The results obtained are presented in figure 7.

These results confirm the conclusion reached above, that as adaptation to darkness progresses, the sensitivity of Peranema

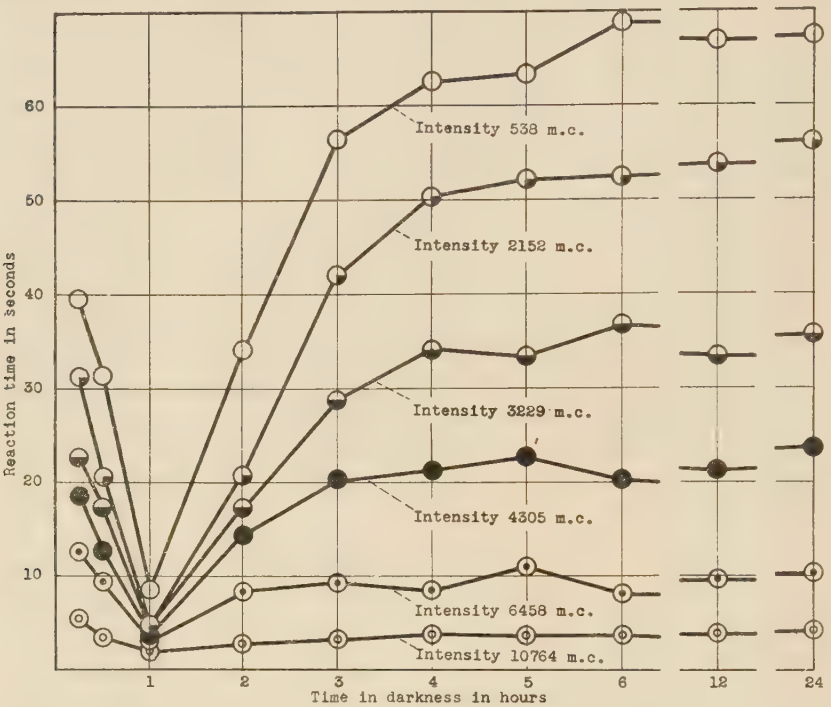

Fig. 6 Graphs showing rate of dark-adaptation. Dark-adapted peranemas were adapted to 2152 m.c. 3 hours, then subjected to darkness for the time indicated, then exposed to light (intensity given in the graph), and the reaction time measured. Each point on the curves represents the average reaction time for ten tests, different individuals being used in each test.

increases to maximum, then decreases to minimum, and then remains nearly constant, and they show that the higher the intensity of the light in which peranemas are exposed for a given length of time, the longer the time in darkness required to neutralize the effect of the light.

RELATION BETWEEN LUMINOUS INTENSITY AND REACTION TIME, EXPOSURE PERIOD, LATENT PERIOD AND ENERGY

In many organisms exposure during the entire reaction time is not necessary in order to obtain a response, i.e., there is a period during which the organism must be exposed, followed by a period during which exposure is not necessary. The time

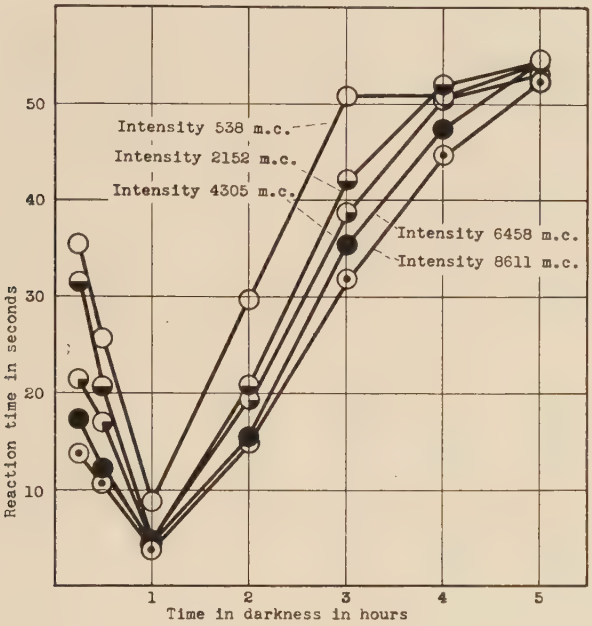

Fig. 7 Graphs showing rate of dark-adaptation. Dark-adapted peranemas were adapted to light (intensity given in the graph) 3 hours, then subjected to darkness for the time indicated, then exposed to 2152 m.c., and the reaction time measured. Each point on the curves represents the average reaction time for ten tests, different individuals being used in each test.

during which exposure is necessary has been termed by different investigators; exposure period, stimulation period, sensitization period, and presentation time; and the time during which exposure is not necessary has been termed latent period. The reaction time consists of the sum of the exposure period and the latent period.

Blauuw ('08), Fröschel ('09), and Clark ('13) conclude that in Avena sativa the product of the intensity of the light and the time during which it must act to produce a response is constant and that the Bunsen-Roscoe law therefore holds. Arisz ('11) contends that it holds for Avena sativa only when bending becomes macroscopically visible. He concludes that the effect of a given amount of light is dependent upon its intensity. Burckhardt ('26) repeated the work of Blauuw and Fröschel on Avena sativa and obtained results which support Arisz.

Ewald ('14) concludes that the amount of light energy necessary to elicit a response by suddenly illuminating the eye of Daphnia pulex is the same over a wide range of intensities. Hecht ('18, '19) in work on Mya arenaria and Ciona intestinalis and Obreshkove ('21) in work on tadpoles of Rana clamitans reach the same conclusion; they assume that the latent period is constant for all intensities and conclude that the exposure period is inversely proportional to the intensity.

Pieron ('26) repeated the observations of Hecht on Mya arenaria and concludes that the amount of light energy required to produce a response is not the same for all intensities, and that the Bunsen-Roscoe law therefore does not hold. Folger ('26) obtained results in observations on Mya arenaria which support the conclusion of Pieron.

Harder ('20) found that if the illumination is rapidly decreased, the hormogonium of Nostoc responds by cessation of movement, followed by movement in the opposite direction. He maintains that the interval between decrease in illumination and reversal in direction of movement is not proportional to the decrease in light intensity.

Results obtained in observations on Amoeba proteus by Folger ('25) show that the reaction time varies inversely with the luminous intensity, but that as the intensity increases, the latent period increases to a maximum and then decreases, and that the amount of light energy required during the exposure period increases to a maximum and then decreases.

Bevelander ('33) reached the same conclusion for Bucephalus elegans.

The apparatus and the methods used in the observations on the relation between luminous intensity and reaction time, exposure period, latent period, and energy in Peranema were essentially the same as those used in the preceding experiments.

TABLE 2

Relation between luminous intensity and exposure period. Each exposure period presented is the result of one observation on one individual, and different individuals were used in each observation

DESIGNATION OF PERANEMAS	LUMINOUS INTENSITY IN METER CANDLES						
	538	1076	2152	3229	4305	5382	6458
	EXPOSURE PERIOD IN SECONDS						
1	40.1	35.2	29.0	12.8	6.8	3.1	2.1
2	44.3	38.3	20.4	13.2	7.2	3.3	1.9
3	43.8	37.6	27.4	11.9	5.9	2.9	2.0
4	47.6	40.2	21.2	14.6	6.6	3.5	2.2
5	39.8	35.9	26.8	14.2	6.9	2.8	2.3
6	42.8	32.1	28.1	10.2	6.8	3.6	1.8
7	48.3	33.8	22.8	13.8	7.0	3.0	2.5
8	41.9	37.5	24.5	14.6	7.1	4.1	1.9
9	38.7	40.2	25.9	15.5	6.3	3.8	2.0
10	39.5	29.8	26.2	9.8	7.5	2.9	2.2
Total average	42.68	37.06	25.23	13.06	6.81	3.29	2.09
Probable error	0.95	0.50	0.56	0.36	0.08	0.08	0.04

The exposure and the latent periods were measured as follows: A dark-adapted specimen was exposed to light of 538 m.c. until it responded; then other dark-adapted specimens were exposed to the same intensity but for successively shorter periods of time until no response occurred, a fresh dark-adapted specimen being used for each test. In this way the shortest exposure which produced a response in this intensity was ascertained. This is the exposure period for the intensity and the specimen used. This was repeated nine times with other specimens exposed to 538 m.c. and ten times with different specimens exposed to 1076, 2152, 3229, 4305,

5382 and 6458 m.c., respectively. Ninety-six meter candles is the lowest intensity in which a response was obtained. The latent period was calculated by subtracting the exposure period from the reaction time for each intensity. The results obtained are presented in table 2 and figure 8.

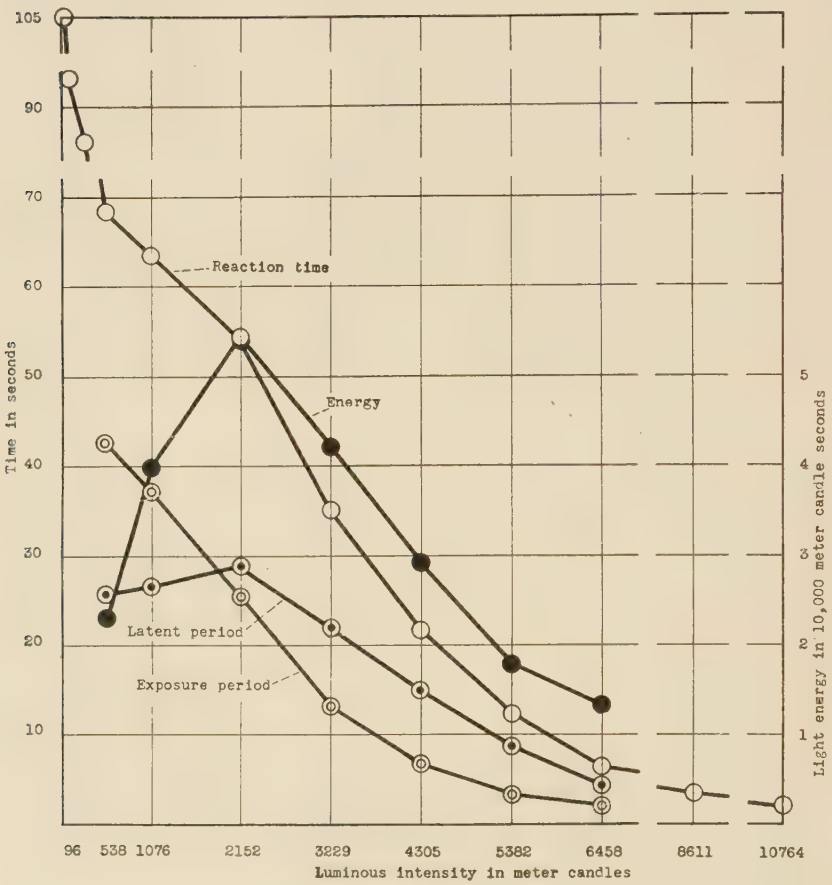

Fig. 8 Graphs showing relation between luminous intensity and reaction time, exposure period, latent period and energy. Each point on the curves of the reaction time and the exposure period represents the average for ten tests, different individuals being used in each test. Each point on the curve of the latent period represents the difference between the reaction time and the exposure period. Each point on the energy curve represents exposure period × luminous intensity.

Table 2 shows that there was remarkably little variation in the exposure period observed in the different individuals used, the probable error being only 0.04 to 0.95.

Figure 8 shows the following: As the luminous intensity increased, the latent period increased from 25.68 seconds at 538 m.c. to a maximum of 28.91 seconds at 2152 m.c. and then decreased to 4.25 seconds at 6458 m.c., the exposure period decreased from 42.68 seconds at 538 m.c., at first rapidly, then more slowly until it became nearly constant, being only 2.09 seconds at 6458 m.c., and the amount of light energy required to induce a response increased from 22,970 m.c. second at 538 m.c. to a maximum of 54,315 m.c. second at 2152 m.c. and then decreased to 13,498 m.c. second at 6458 m.c.

The results therefore prove that the amount of light energy required to induce a shock reaction in Peranema varies greatly with the intensity of the light and that the Bunsen-Roscoe law consequently does not hold.

RELATION BETWEEN TEMPERATURE AND REACTION TIME, EXPOSURE PERIOD AND LATENT PERIOD

Hecht ('18, '19, '27) maintains that in Mya arenaria, Ciona intestinalis, and Pholas dactylus the latent period varies inversely with temperature, and that the exposure period is nearly independent of temperature. Cole ('22) asserts that in Necturus maculosus the photic reaction time varies directly with temperature. Folger ('25) concludes that in Amoeba proteus temperature apparently has little if any effect on the reaction time. Bevelander ('33) concludes that the reaction time in Bucephalus elegans varies inversely with temperature.

In the observations on the relation between temperature and reaction time, exposure period, and latent period in Peranema, a Pitts ('32) constant temperature bath was used in addition to the apparatus described above. This bath consists essentially of a brass chamber and a Pyrex glass cup. Water of known temperature was circulated through the chamber and around the cup so as to maintain the temperature in the cup constant. It was placed on the stage of

the microscope and transmitted light for observation was received through a glass window in the bottom.

The observations were made as follows: A dark-adapted specimen was mounted on the bottom of the glass cup in a drop of filtered culture fluid under a cover glass supported with a vaseline ring. By means of the bath the temperature was maintained at 10° ½ hour, after which the specimen was suddenly exposed to 2152 m.c., and the reaction time measured.

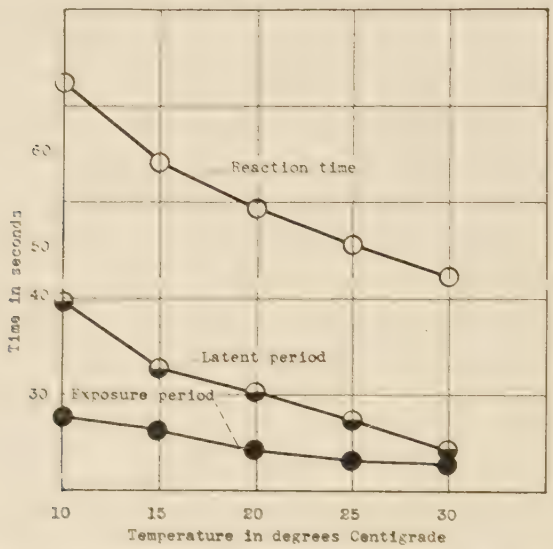

Fig. 9 Graphs showing relation between temperature and reaction time, exposure period, and latent period. Each point on the curves of the reaction time and the exposure period represents the average for ten tests, different individuals being used in each test. Each point on the curve of the latent period represents the difference between the reaction time and the exposure period.

This process was repeated nine times with other specimens. Then the exposure and the latent periods at this temperature were ascertained as described above. Then the reaction time, exposure period, and latent period were ascertained in the same way at 15, 20, 25 and 30°, respectively. The results obtained are given in figure 9.

It will be seen from this figure that as the temperature increased, the exposure period decreased from 27.87 seconds

at 10° to 22.97 seconds at 30°, while the latent period decreased from 39.68 seconds at 10° to 24.30 seconds at 30°.

The results presented indicate that there are at least two processes involved in the response of Peranema to light, one which is nearly independent of temperature and is therefore probably photochemical, and another which is closely correlated with temperature and is therefore not photochemical.

RELATION BETWEEN LUMINOUS INTENSITY AND THE RECOVERY PERIOD

The time, after a response, required for an organism to attain its former physiological state is designated the recovery period. Folger ('25) observed that if Amoeba proteus has responded to sudden increase in light intensity, a period of time, ranging from 1 to 2 minutes must elapse before a second response to sudden increase in intensity can occur, i.e., that for amoeba the recovery period, after response to sudden increase in illumination, is 1 to 2 minutes. He found that during the first part of this period the amoeba may remain either in light of the same intensity as that which induced the response, or in lower intensity, or in darkness, but that during the second part, ranging from 10 to 20 seconds, it must be in darkness or in lower intensity than that which produced the response.

Two methods were used in ascertaining the recovery period for Peranema.

1. A dark-adapted specimen was suddenly exposed to 2152 m.c., and the reaction time measured as described above. Then it was subjected to darkness 3 seconds, after which it was again exposed, and the reaction time measured. Then it was subjected to darkness 5 seconds, after which it was again exposed, and the reaction time measured. This was now repeated with the period in darkness 10, 15, 30, 45, 60, 90, and 120 seconds respectively, after which the specimen was discarded. Then the whole process was repeated with three other specimens exposed in the same intensity and with eight other specimens, four exposed in 4305 m.c. and four

exposed in 6458 m.c. The results obtained are presented in figure 10.

This figure shows that the reaction time of fully dark-adapted peranemas was 50.50 seconds in 2152 m.c., 21.55 seconds in 4305 m.c., and 7.84 seconds in 6458 m.c., that the reaction time of the same specimens after dark-adaptation of 3 seconds following the response to light was only 22.47 seconds

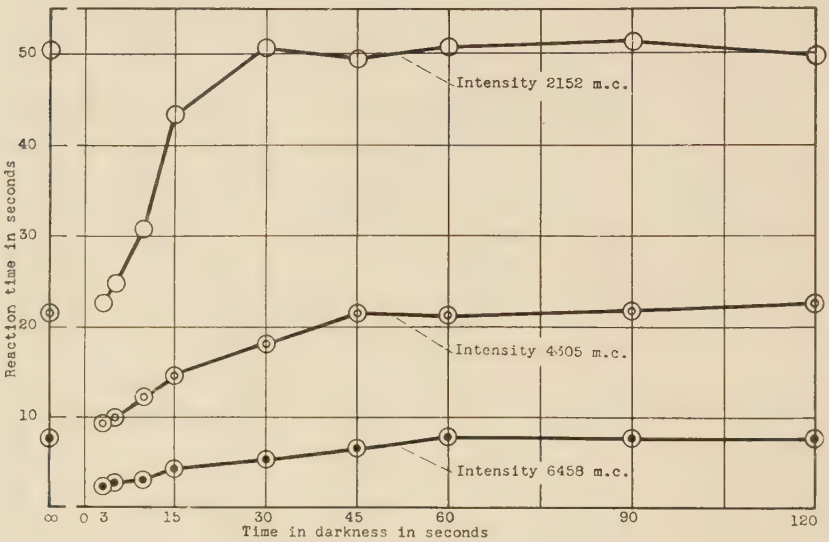

Fig. 10 Graphs showing rate of recovery in darkness after response to light of different intensities. Dark-adapted peranemas were exposed to light (intensity given in the graph), and the reaction time measured. They were then kept in darkness for the time indicated, after which they were again exposed, and the reaction time measured. Each point on the curves represents the average reaction time for four tests, different individuals being used in each test.

in 2152 m.c., 9.36 seconds in 4305 m.c., and 2.33 seconds in 6458 m.c., and that as dark-adaptation increased, the reaction time increased until it was the same as it had been initially, but that the time in darkness required for this was 30± seconds after response in 2152 m.c., 45± seconds after response in 4305 m.c., and 60± seconds after response in 6458 m.c., i.e., that the time required for recovery varied directly with the intensity of the light which induced the response.

2. A dark-adapted specimen was suddenly exposed to 6458 m.c., and left until it responded plus 5 seconds. Then it was subjected to darkness 15 seconds, after which it was again exposed and left until it responded plus 10 seconds. This was repeated six times with this specimen but with the time in light after the response 15, 30, 45, 60, 90 and 120 seconds, respectively. The whole process was then repeated three times with other specimens. The results obtained are presented in figure 11.

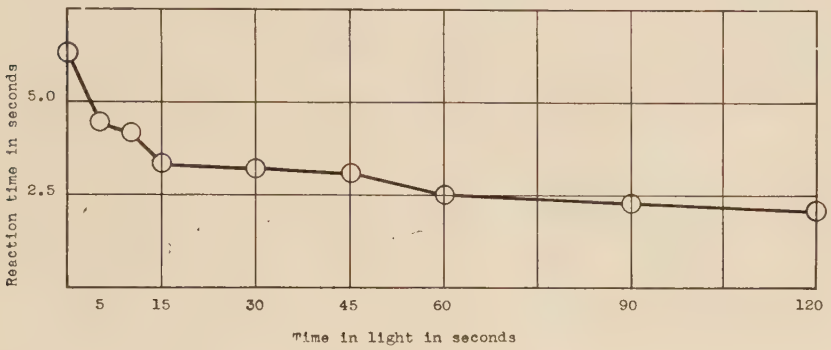

Fig. 11 Graph showing effect of adaptation to light on the reaction time. Dark-adapted peranemas were exposed to 6458 m.c., and the reaction time measured. They were then kept in the light for the time indicated and in darkness 15 seconds, after which they were again exposed, and the reaction time measured. Each point on the curve represents the average reaction time for four tests, different individuals being used in each test.

This figure shows that as the time the peranemas were in the light increased, the reaction time decreased until it reached a minimum and then remained nearly constant, i.e., that recovery did not occur while they were in light of the same intensity as that used to induce the response.

RELATION BETWEEN TEMPERATURE AND THE RECOVERY PERIOD

The recovery period for Peranema after response to 6458 m.c. at 15, 20 and 25°, respectively was ascertained as described above. The results obtained are presented in figure 12.

Figure 12 shows that in 6458 m.c. the reaction time of fully dark-adapted peranemas was 10.21 seconds at 15°, 8.12 seconds at 20°, and 6.20 seconds at 25°, that after 3 seconds in darkness following this response, the reaction time of the same specimens was only 4.52 seconds at 15°, 2.16 seconds at 20°, and 1.70 seconds at 25°, and that as the time in darkness following the response increased, the reaction time increased

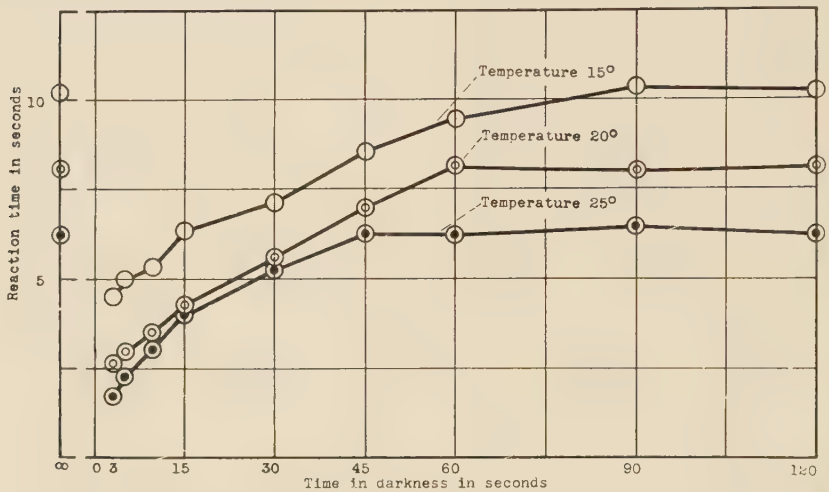

Fig. 12 Graphs showing effect of temperature on the rate of recovery in darkness after response to light of a given intensity. Dark-adapted peranemas were exposed to 6458 m.c. (at the temperature given in the graph), and the reaction time measured. They were then kept in darkness for the time indicated, after which they were again exposed, and the reaction time measured. Each point on the curves represents the average reaction time for four tests, different individuals being used in each test.

until it was the same as it had been initially, and the organisms had consequently fully recovered from the effect of exposure to light. The figure also shows that the time in darkness required for full recovery was 90± seconds at 15°, 60± seconds at 20°, and 45± seconds at 25°.

The results presented show therefore that the time in darkness required for recovery after response to light of a given intensity varies inversely with the temperature.

EFFECT OF DRUGS ON THE SENSITIVITY TO LIGHT

The observations on the effect of drugs on the sensitivity of Peranema to light were made as follows: A dark-adapted specimen was mounted on each of three slides in a drop of 0.1% strychnine sulphate in distilled water under a cover glass supported with a vaseline ring. Then the slides were put into a light-tight box. After the specimens had been in the drug 1 hour, one was suddenly exposed to 2152 m.c., and the reaction time measured as described above. This was repeated with the other specimens after having been in the drug 3 and 6 hours, respectively. This process was then repeated with the concentration of the strychnine sulphate 0.01, 0.001, 0.0001 and 0.00001%, respectively. The whole

TABLE 3

The effect of cocaine chloride and strychnine sulphate on the sensitivity to light in Peranema trichophorum. Each number represents the average for ten tests with dark-adapted specimens in 2152 m.c., different individuals being used in each test

CHEMICALS	TIME IN SOLUTION IN HOURS		
	1	3	6
	REACTION TIME IN SECONDS		
Distilled water	54.31	53.27	53.48
Cocaine chloride 0.0001%	69.54	70.89	68.59
Strychnine sulphate 0.0001%	6.82	6.30	6.99

process was repeated with cocaine chloride in place of strychnine sulphate.

The results obtained showed that the optimum concentration of strychnine sulphate and cocaine chloride is approximately 0.0001%.

Then the above process was repeated ten times with other specimens in optimum concentration of strychnine sulphate and cocaine chloride, respectively, and in distilled water. The results obtained are presented in table 3.

This table shows that the reaction time in 2152 m.c. was independent of the length of time the peranemas were in the different solutions, but that it was 54± seconds for those in distilled water, 69± seconds for those in distilled water

plus cocaine chloride, and only 7± seconds for those in distilled water plus strychnine sulphate. Figure 8 shows that the reaction time in 2152 m.c. is 54± seconds for specimens in culture fluid. The cocaine chloride consequently caused an increase in the reaction time of about 28% and the strychnine sulphate a decrease of about 87%.

The results therefore show that strychnine sulphate causes great increase and cocaine chloride considerable decrease in the sensitivity to light.

DISCUSSION

Mast ('07) postulated a reversible photochemical reaction to explain reversal in the sense of photic orientation in Volvox globator in which he assumed the following: Two substances, X and Y, the chemical reaction between which is dependent upon the luminous intensity; with increase in intensity there is increase in Y and decrease in X; a sub-optimum intensity or darkness favors the formation of X and a supra-optimum of Y; the colonies are neutral in reaction if there are Y substances in one member of the equation and X in the other, positive if one member contains X+ substances and the other Y—, and negative if one member contains X— and the other Y+.

In 1927 he modified this hypothesis by postulating two additional substances, M and N, which are non-photochemical in nature but which react with X and Y in such a way as to account for additional facts concerning reversal in orientation.

In 1932 he says "When *Volvox* is photopositive a shadow on the photosensitive substance in the eye causes a change in the direction of the stroke of the flagella from diagonal to straight backward and a flash on this substance causes a change in the stroke from diagonal to sidewise, whereas when *Volvox* is photonegative, precisely the opposite obtains." In order to account for this, he postulated another set of photochemical substances, O and P, which are associated with X, Y, M, and N in such a way that in photopositive colonies a shadow produces an increase in O, and a flash an increase in

P, whereas in photonegative colonies the reverse occurs. Thus, three sets of interacting substances are postulated in order to account for the response to light in Volvox.

Hecht ('18, '19, '27) formulated a theory to account for photic responses in invertebrates and vision in man. This theory is similar to that formulated by Mast. It follows: The photosensitive mechanism consists of two processes, a photochemical reaction, independent of temperature, and a chemical reaction, dependent upon temperature. In the former, a photosensitive substance, S, is decomposed by the action of light, forming two substances, P and A, which catalyze an inert substance, L, changing it into a chemically active substance, T, which in turn acts on the nerve to initiate the sensory impulse. In the dark P and A unite and form the original substance S. The photochemical reaction is consequently reversible.

As to whether there are chemical processes associated with the response to light in Peranema similar to those formulated by Mast or Hecht, no definite conclusions can be drawn, but some of the results show clearly that there are at least two processes involved in producing the response, one which is dependent upon light and is reversible, and another which is independent of light. This is demonstrated by the following facts:

1. After a peranema has responded to increase in illumnination, it must be in darkness in order to recover from the effect of the light before it will respond again, and the greater the intensity of the light the longer the time in darkness required to neutralize its effect.

2. In order to produce a response the illumination must continue for a given part of the reaction time, the exposure period, and it need not continue for the remainder of the reaction time, the latent period.

3. As the temperature increases, the exposure period decreases, but only slightly, while the latent period decreases considerably.

4. The time in darkness required for recovery after response to light varies directly with the luminous intensity and inversely with the temperature.

These processes can be accounted for by the theories considered above, but the following facts cannot: The latent period and the amount of light energy required to induce a response vary greatly with the intensity of the light: as fully dark-adapted peranemas become light-adapted and fully light adapted peranemas become dark-adapted, the sensitivity increases to a maximum, then decreases to a minimum, and then remains nearly constant. I can offer no satisfactory explanation for these facts.

SUMMARY

1. If the illumination is rapidly increased, Peranema responds with rapid deflection of the anterior end. If it is slowly increased, this does not occur. This response therefore depends upon the rate of change in luminous intensity and consequently is a typical shock reaction.

2. The entire organism is sensitive to light, the flagellum most, the anterior end less, and the posterior end least. There is no indication whatever of a differential response to localized stimulation.

3. The detached flagellum responds to increase in illumination by bending, especially at the tip, but it does not recover.

4. The maximum stimulating efficiency in the visible spectrum is at wave length 505 ± mμ. Stimulation by light is dependent upon the wave length as well as upon the intensity, and the limits of the spectrum are dependent upon the intensity.

5. As light-adaptation increases, the reaction time decreases to a minimum in about ½ hour, then increases to a maximum in about 3 hours more, and then remains nearly constant, i.e., the sensitivity to light increases to a maximum, then decreases to a minimum, and then remains nearly constant.

6. As dark-adaptation increases, the reaction time decreases to a minimum in about 1 hour, then increases to a maximum in about 3 hours, and then remains nearly constant, i.e., the sensitivity to light increases to a maximum, then decreases to a minimum, and then remains nearly constant.

7. The greater the intensity to which peranemas are fully adapted, the longer the time in darkness required to neutralize the effect of the light.

8. The reaction time consists of an exposure period and a latent period. With increase in intensity of illumination, the exposure period decreases at first rapidly, then more slowly until it becomes nearly constant, the latent period increases to a maximum and then decreases, and the amount of light energy required during the exposure period increases from 22,970 m.c. seconds at 538 m.c. to 54,315 m.c. seconds at 2152 m.c. and then decreases to 13,498 m.c. seconds at 6458 m.c.

9. With increase in temperature, the latent period decreases considerably, but the exposure period decreases only slightly.

10. There are at least two processes involved in the response to light, one of which is probably photochemical.

11. The time in darkness required for recovery after response to light varies directly with the luminous intensity in which the response occurs and inversely with the temperature.

12. Recovery does not occur while peranemas are in light of the same intensity as that used to induce the response.

13. Strychnine sulphate causes great increase and cocaine chloride considerable decrease in the sensitivity to light.

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VITA

. Landrum Brewer Shettles was born in Pontotoc County, Mississippi, November 21, 1909. He attended the Friendship public school in that county and was graduated from Ecru High School, Ecru, Mississippi, in 1930, being valedictorian of his class. He spent the summer of 1930 at the University of Texas, Austin, and the next 3 years at Mississippi College, Clinton, where in August, 1933, he received the degree of Bachelor of Arts (Special Distinction). In 1930–1931 he was the recipient of a scholarship to Mississippi College, and during the year 1932–1933 he was student assistant in general zoölogy. In the fall of 1933 he entered the graduate school at the University of New Mexico, Albuquerque, from which he received the degree of Master of Science in biology in 1934. He was assistant in general zoölogy and bacteriology in 1933–1934. During the summer of 1934 he was employed by the U. S. Bureau of Fisheries as assistant biologist in a survey of streams and lakes of the Carson and Sante Fe National Forests of New Mexico. In the fall of 1934 he entered the School of Higher Studies in the Johns Hopkins University where he held a university scholarship in 1934–1935. He was assistant in comparative anatomy and embryology in 1935–1936, and assistant in general biology in 1936–1937. He spent the summer of 1935 in Woods Hole, Massachusetts, where, with the aid of a scholarship from the Johns Hopkins University, he took the course in invertebrate zoölogy at the Marine Biological Laboratory and spent the latter part of the summer in research under the direction of Dr. S. O. Mast. This research was continued at the Johns Hopkins University as the subject of his dissertation. He was elected an active member of Sigma Xi and Gamma Alpha in 1937, and awarded the Bruce Fellowship in zoölogy for 1937–1938. During the summer of 1937 he worked as junior aquatic biologist for the Institute for Fisheries Research, Ann Arbor, Michigan, in cooperation with the Michigan Department of Conservation and the U. S. Forest Service, in a survey of lakes of the Manistee, Huron, Hiawatha and Ottawa National Forests of Michigan.